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TITLE: Phosphatidylinositol 3-Kinase and Protein Kinase C as Molecular Determinants of Chemoresistance in Breast Cancer

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13. ABSTRACT ***(Maximum 200 Words)***

The goal of this project is to identify novel therapeutic strategies aimed at counteracting or reversing drug resistance in breast cancer. Chemotherapeutic drug resistance may result, in part, from a shift in the regulation of cellular mechanisms away from apoptosis to a more survival-oriented pathway. Two proteins that have been implicated as anti-apoptotic are protein kinase C and phosphatidylinositol 3-kinase. Although differential expression of these kinases have been linked to anti-apoptotic signaling mechanisms, the molecular details of upstream and downstream events are not well understood, and therefore elucidation of their mechanisms of action may represent a potential therapeutic target for breast cancer. Using an isogenic model system of estrogen receptor positive, apoptosis-sensitive and apoptosis-resistant breast cancer cell variants, this proposal aims to define the role of phosphatidylinositol 3-kinase and specific protein kinase C isoforms in cellular apoptotic signaling pathways. We have found that PKC α and δ isoforms are differentially expressed in our isogenic model. We are currently optimizing the use of green fluorescent protein-tagged, as well as constitutive-active and dominant-negative, PKC constructs, in order to better understand how PKC and PI3K may affect survival and apoptotic signaling in breast cancer.

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ANNUAL REPORT

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Award Number: DAMD17-01-1-0432

INTRODUCTION

The goal of this project is to identify novel therapeutic strategies aimed at counteracting or reversing drug resistance in breast cancer. Chemotherapeutic drug resistance, or failure to initiate apoptosis as a response to a chemotherapeutic drug, may result, in part, from a shift in the regulation of cellular mechanisms away from apoptosis to a more survival-oriented pathway. A diminished susceptibility to apoptosis may be mediated by the differential expression certain key proteins, which serve as molecular determinants for the cancer cell's capacity to survive many environmental stresses, including chemotherapeutic drug treatments. Two proteins that have been implicated as anti-apoptotic are protein kinase C and phosphatidylinositol 3-kinase. Although differential expression of these kinases have been linked to anti-apoptotic signaling mechanisms, the molecular details of upstream and downstream events are not well understood, and therefore elucidation of their mechanisms of action may represent a potential therapeutic target for breast cancer. Using an isogenic model system of estrogen receptor positive, apoptosis-sensitive and apoptosis-resistant MCF-7 breast cancer cell variants, this proposal aims to define the role of phosphatidylinositol 3-kinase and specific protein kinase C isoforms in cellular apoptotic signaling pathways. It is expected that a detailed understanding of the role of these two kinases in breast cancer cell anti-apoptotic signaling pathways will provide opportunities for novel pharmacological interventions in *in vivo* model systems.

ANNUAL REPORT

Amanda P. Parker

Award Number: DAMD17-01-1-0432

Statement of Work: To test the hypothesis that PI3K and PKC are molecular determinants of chemoresistance in breast cancer

Task 1. Determine relationship between PI3K/Akt expression, PKC isoform expression, and cell survival

Task 2. To characterize the role of PKC isoform expression in apoptosis-sensitive (MCF7-N) breast cancer cells and in apoptosis-resistant (MCF7-TN) cells.

Task 3. Determine the specific mechanism for PKC-mediated breast cancer cell survival or chemoresistance.

The goal of this study is to investigate the role of PI3K and PKC in chemoresistance in breast cancer. I began my studies using a previously published model system using isogenic MCF-7 cell variants, (MCF-7 N (apoptosis-sensitive) and MCF-7 M (apoptosis-resistant), which exhibited differential sensitivity to TNF-α and other chemotherapeutic drugs. While the MCF-7 M variant was less sensitive to TNF-α than the N variant, it still was partial susceptible to the cytotoxic effects of TNF-α. In order to have a more complete model of resistance, and to more effectively investigate the role of PI3K and PKC in MCF-7 breast cancer cell survival, this lab has developed an MCF-7 variant (MCF-7 TN) which exhibits nearly complete resistance to TNF-α, from the MCF-7 N (apoptosis-sensitive) variant. The resistance of this variant was confirmed using trypan blue viability, and long-term, clonogenic assays (Appendix, Figure One).
I have used immunoblotting in this MCF-7 model system to investigate which PKC isoforms have are altered between the MCF-7 N and MCF-7 TN variants. I have implicated PKC-α and PKC-δ as potential determinants in MCF-7 cell variant resistance and survival, as these studies revealed an upregulation in the expression and phosphorylation of PKC-α, with downregulation of PKC-δ, with no detectable change in PKC-ξ (Appendix, Figure Two). While our laboratory has previously implicated PI3K-Akt as a survival factor in MCF-7 cell variants, I did not detect any significant changes in expression or phosphorylation of Akt in the N vs. TN variant. However, Akt activity assays have not yet been performed.

I have obtained genetic constructs for green fluorescent protein (GFP)-tagged PKC isoforms α, β, δ, ε, γ, η, θ, and ξ, and have been able to transfect and detect the α, β, γ, η, θ, and ξ constructs with good efficiency (Appendix, Figure Three). However, I have had difficulty with constructs obtained for GFP-PKC δ and ε, and have not been able to detect these constructs with fluorescent microscopy. I am currently working on either obtaining new constructs for these isoforms. In addition, I have obtained and produced constructs for constitutive active (CA) and dominant negative (DN) PKC α, β, δ, ε, θ, and ξ, and am in the process of optimizing the use of these constructs.

Last, I am using luciferase reporter gene assays to characterize the changes in NF-κB transcriptional activity in the MCF-7 TN variant versus the MCF-7 N variant. This laboratory has previously shown that the PI3K-Akt- NF-κB pathway is an important survival pathway in MCF-7 cells, Consistent with this, luciferase reporter gene assays have shown that the NF-κB pathway is dramatically up-regulated in the TN variant as compared to the N variant in response to TNF-α treatment (Appendix, Figure Four). Binding of TNF-α to its cell surface receptor can activate both survival and apoptotic responses, depending on the cell, many of which are dependent on activation of NF-κB. That NF-κB transactivation is increased to such a degree in the TNF-α-resistant MCF-7 TN variant suggests that, in these cells, NF-κB is play an important role in TNF-α resistance.

KEY ACCOMPLISHMENTS

- Development of the MCF-7 TN variant as a better model system to investigate mechanisms of breast cancer cell resistance
- Characterization of key proteins and kinases up- and downregulated in MCF-7 TN cell variant
- Optimization of GFP-PKC isoform constructs
- Implication of NF-κB pathway in MCF-7 TN cell variant resistance to TNF-α

OUTCOMES

- Development of TNF-α-resistant MCF-7 TN clone
- Presentation of data at Tulane Health Science Center Research Days (April, 2002), XIV International World Congress of Pharmacology (July, 2002)

CONCLUSIONS

The development of resistance to standard chemotherapeutic protocols is a major obstacle to successful treatment of almost a type of cancer. A complete and thorough understanding of how resistance develops in breast cancer cells is needed to more effectively treat this disease. Our MCF-7 breast cancer model system will be useful in determining how two known survival pathways affect one another in determining chemoresistance.

APPENDIX

(A) (B)

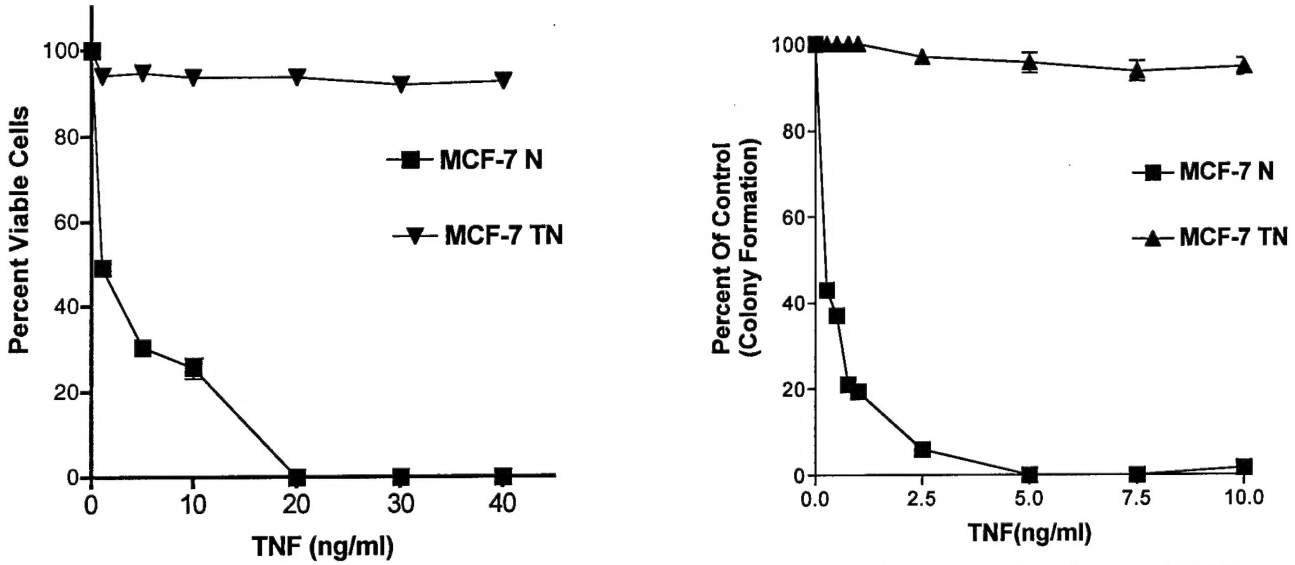


Figure One: **(A)** MCF-7 N and TN cells were treated with increasing doses of TNF-α for 48 h, and viability was determined by trypan blue exclusion. **(B)** MCF-7 N and TN cells were treated with the indicated doses of TNF for 24h, followed by an eight day incubation period. Colonies over 1mm in diameter were counted as positive.

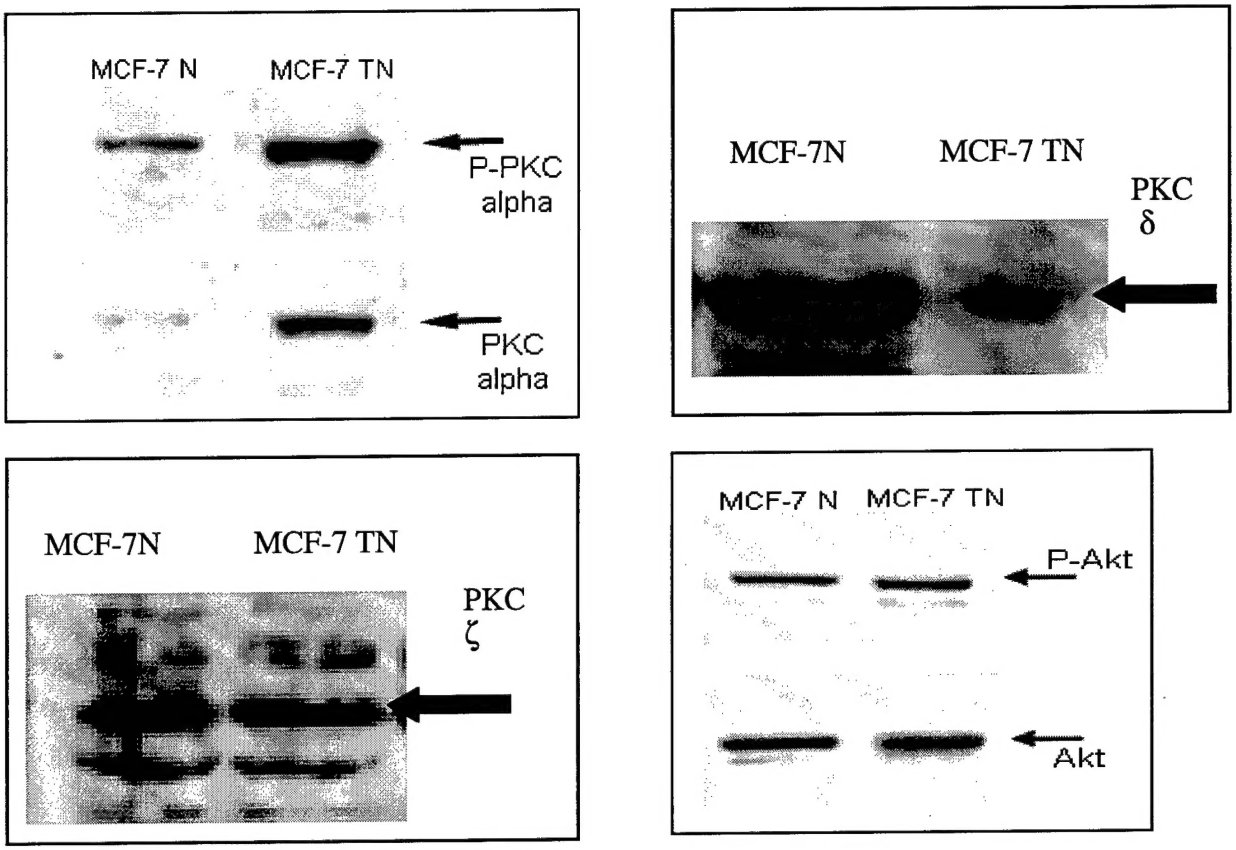
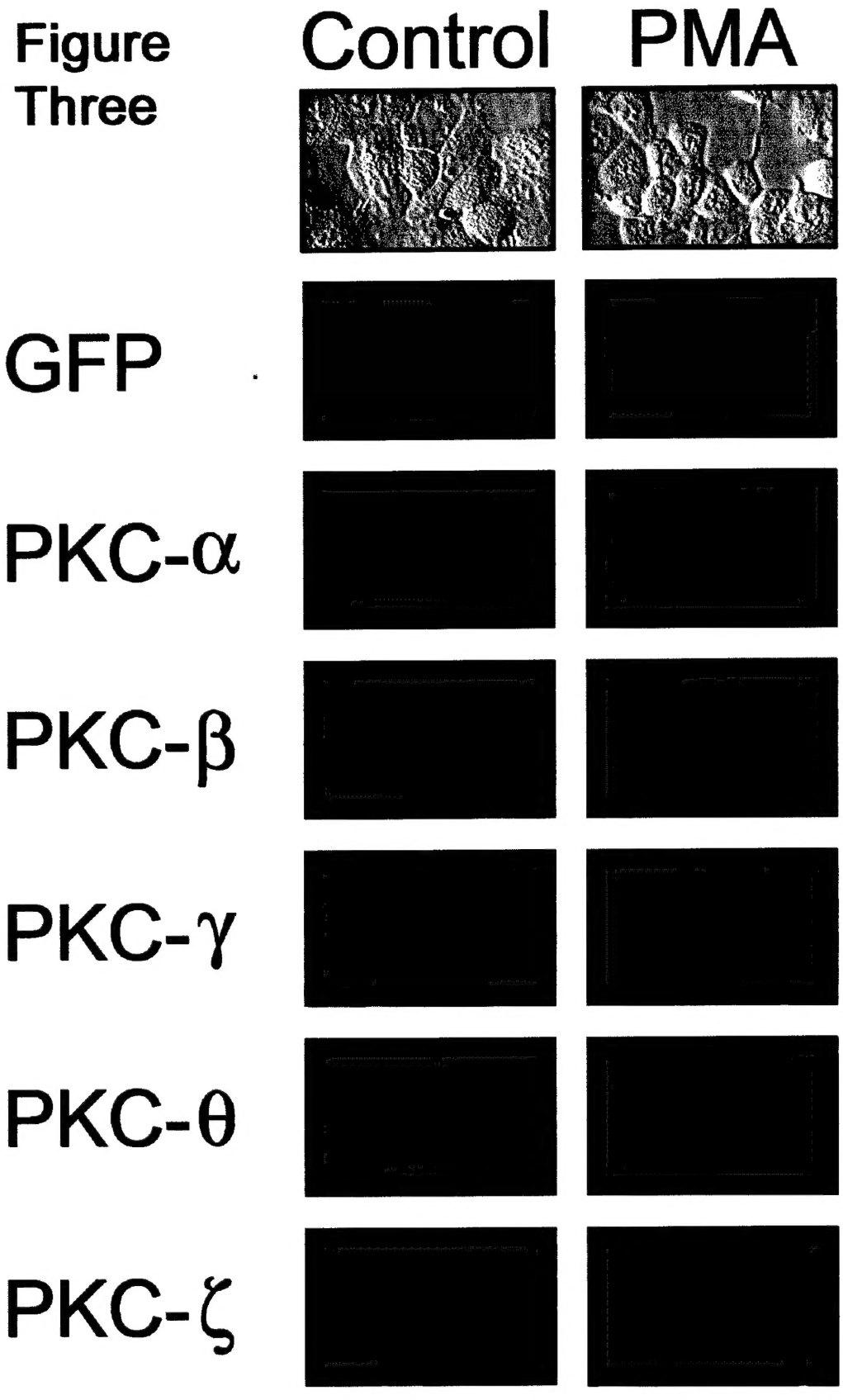


Figure Two: To investigate which signal transduction pathways might be involved in he MCF-7 TN cell variant resistance, Western blot analysis was performed on the MCF-7 N and TN cells. MCF-7 N and TN cell lysates were electrophoresed, transferred onto nitrocellulose membranes, and probed with antibodies for the indicated proteins.

Figure Three



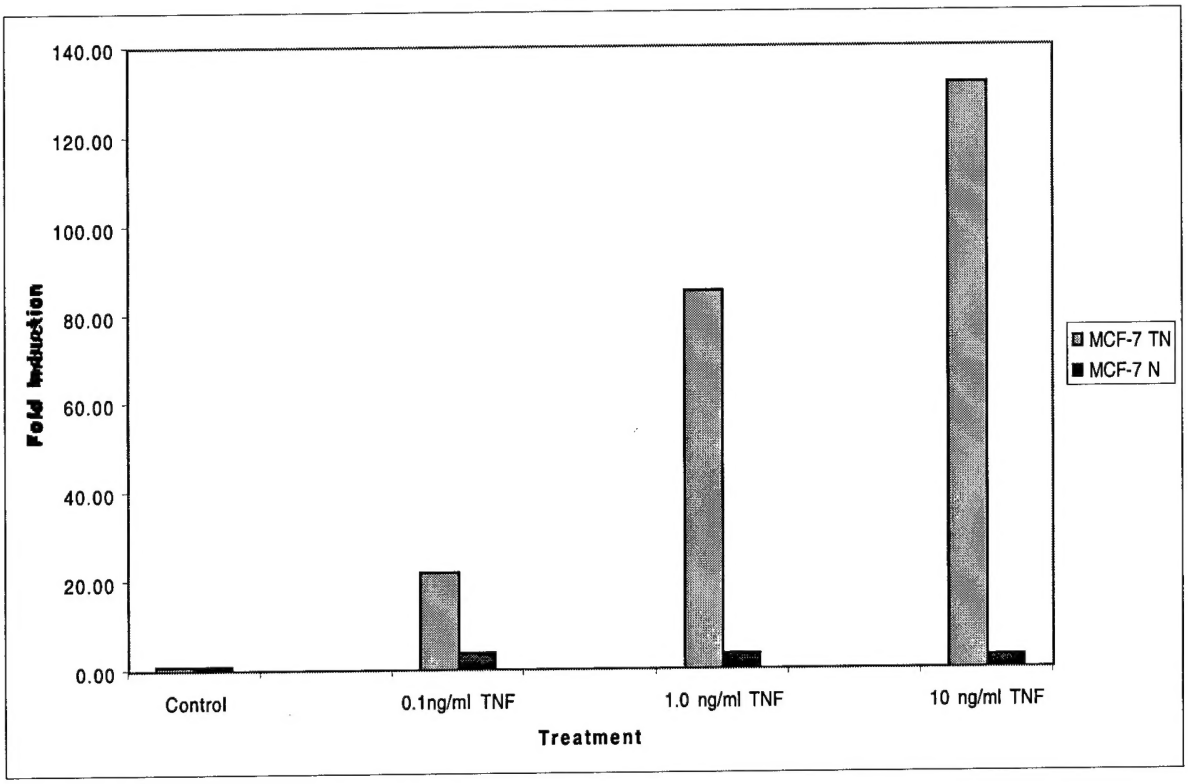


Figure Four: MCF-7 TN cells were transiently transfected with a NF-kB-luciferase reporter gene construct overnight before the indicated treatments for 16 hours. Results are expressed as fold induction over vehicle treated control. Results demonstrate that TNF-α stimulates NF-κB to a much greater extent in the MCF-7 TN cells versus the MCF-7 N cells, and supports the hypothesis that NF-κB plays a role in MCF-7 cell variant resistance to TNF- α

Amanda P. Parker
DAMD17-01-1-0432

SUMMARY OF TRAINING (7/01/2001 – 6/30/2002)

- Attended weekly seminars concerning and clinical developments in the Tulane Cancer Center.
- Attended weekly seminars with visiting speakers in the Tulane University Department of Pharmacology.
- Acquired significant teaching experience in two classes, Drugs and Their Actions and Medical Pharmacology, both of which contain subject blocks on cancer chemotherapy.
- Presentation of data at Tulane University Research Days (April 2002) and XIV World Congress of Pharmacology (July 2002).
- Completion of all required classes, as well as successful completion of departmental Preliminary Exams.
- Learned a variety of laboratory techniques, including MTT viability assays, immunoblotting, luciferase reporter gene assays, polymerase chain reaction, fluorescent microscopy, mitochondrial membrane potential assays, and statistical software programs.